

ABSTRACT

This invention provides a method for deriving precursors to pluripotent non-embryonic stem (P-PNES) and pluripotent non-embryonic stem (PNES) cell lines. The present invention involves nuclear transfer of genetic material from a somatic cell into an enucleated, zona pellucida free human ooplastoid having a reduced amount of total cytoplasm. The present invention provides a new source for obtaining human and other animal pluripotent stem cells. The source utilizes as starting materials an oocyte and a somatic cell as the starting materials but does not require the use, creation and/or destruction of embryos or fetal tissue and does not in any way involve creating a cloned being. The oocyte never becomes fertilized and never develops into an embryo. Rather, portions of the oocyte cytoplasm are extracted and combined with the nuclear material of individual mature somatic cells in a manner that precludes embryo formation. Murine, bovine, and human examples of the procedure are demonstrated. Subsequently, the newly constructed P-PNES cells are cultured *in vitro* and give rise to PNES cells and cell colonies. Methods are described for culturing the P-PNES cells to yield purified PNES cells which have the ability to differentiate into cells derived from mesoderm, endoderm, and ectoderm germ layers. Methods are described for maintaining and proliferating PNES cells in culture in an undifferentiated state. Methods and results are described for analysis and validation of pluripotency of PNES cells including cell morphology, cell surface markers, pluripotent tumor development in SCID mouse, karyotyping, immortality in *in vitro* culture.

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